

EXHIBIT K – ANIMAL EUTHANASIA POLICY

Approved and Adopted: 2015

Last Revised: 12/15/20



Animal Euthanasia Policy

IACUC Policy:

Euthanasia methods for laboratory animals must be consistent with the most recent edition of the American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals.

Exceptions to these standards must be described in the protocol, scientifically justified, and approved by the IACUC.

For all methods, care must be taken to ensure that euthanasia is complete and that the animal(s) cannot recover.

Personnel using physical methods of euthanasia on conscious animals must be well trained and must demonstrate proficiency for each type of physical method performed (i.e., cervical dislocation or decapitation) to ensure euthanasia

Guidelines:

The following tables include methods of euthanasia that are consistent with the AVMA euthanasia guidelines for common species used at Washington University. For all other species, please contact DCM for guidance.

Animal Species	Method of Euthanasia
Large Animals Dog Cat Ferret Sheep Goat Pig Monkey Rabbit	<ol style="list-style-type: none"> 1. Pentobarbital overdose $\geq 150\text{mg/kg}$ IV (Commercial Euthanasia Solutions contain 390 mg/ml pentobarbital) <ol style="list-style-type: none"> a) Beuthanasia® or Euthasol® (also contain 50 mg/ml phenytoin) b) Fatal-Plus® (does not contain sodium phenytoin) 2. General anesthesia followed by: <ol style="list-style-type: none"> a) Perfusion (terminal surgery) or exsanguination b) Vital organ removal c) IV or IC Potassium Chloride (KCl) injection (75-150 mg/kg or 1-2 mEq K⁺/kg) 3. General anesthesia or deep sedation/tranquilization followed by carbon dioxide inhalation <ol style="list-style-type: none"> a) This method can only be used in rabbits

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Animal Species	Method of Euthanasia
Rodents Mouse (> 7 days old) Rat (> 7 days old) Hamster (> 7 days) Gerbil (> 7 days) Guinea Pig (all ages) Chinchilla (all ages)	<p>Acceptable methods</p> <ol style="list-style-type: none"> 1. Anesthetic agents—lethal doses of anesthetics or euthanasia solution <ol style="list-style-type: none"> a. Pentobarbital ≥ 150 mg/kg IP or IV b. Ketamine/Xylazine (or equivalent) 5 x anesthetic dose (e.g., ket/xyl at 500/50 mg/kg IV or IP) c. Inhalant anesthetic overdose (e.g., isoflurane, sevoflurane) 2. Carbon Dioxide (compressed gas) delivered at 30-70% chamber volume displacement per minute, using either: <ol style="list-style-type: none"> a. SmartBox—delivers appropriate rate and sufficient exposure when used as directed b. Other CO₂ systems—need flow meter to control delivery and system must deliver 30-70% chamber volume displacement. This flow rate should be prominently posted in the area where this activity is being done; must continue exposure for > 1 minute following cessation of breathing to ensure death 3. General anesthesia followed by physical method: <ol style="list-style-type: none"> a. perfusion (terminal surgery), exsanguination, or removal of other removal of vital organs/tissues (heart, brain, etc.) b. decapitation, cervical dislocation, or thoracotomy <p>Acceptable with conditions: Cervical dislocation or decapitation is acceptable without anesthesia on conscious rodents when scientifically justified and under the following conditions:</p> <ol style="list-style-type: none"> 1. Animal weight is less than 200 grams. 2. Only trained personnel perform the procedure.
Neonatal rodents (≤ 7 days old) Mouse neonates Rat neonates Hamster neonates Gerbil neonates	<p>Neonates ≤ 7 days old of altricial rodent species may be euthanized by:</p> <ul style="list-style-type: none"> • Decapitation (no prior treatment required) • Injectable anesthetic overdose • Inhalant anesthetic overdose followed by removal of vital organ or decapitation

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Animal Species	Method of Euthanasia
Amphibians Aquatic frogs (Xenopus) Terrestrial frog (Rana) Toads Salamanders Reptiles Turtles Snakes Lizards	Two-step euthanasia protocol: <ol style="list-style-type: none"> Step one—General anesthesia with one of the following: <ol style="list-style-type: none"> Pentobarbital or euthanasia solution MS-222 in water bath or direct injection Dissociative agent combinations: Ketamine/Xylazine or Telazol (drugs injection sites are into dorsal lymph sac or coelomic cavity) Inhaled Agents: Isoflurane Step two—Use one of the following physical methods to ensure death: <ol style="list-style-type: none"> Pithing—must be performed by properly trained individuals Exsanguination or perfusion Removal of vital organ Three-step euthanasia protocol: <ol style="list-style-type: none"> Step one—General anesthesia (see above) Step two—Decapitation Step three—Pithing. The CNS of reptiles and amphibians is tolerant to hypoxia and hypotension, so decapitation must be followed by a method to rapidly destroy brain tissue.

Animal Species	Method of Euthanasia
Fish	Adult Fish (all species/sizes) <ul style="list-style-type: none"> Anesthetic overdose—Tricaine methane sulfonate* in a 0.05-1% solution (500 mg-10 g /L) for a minimum of 10 minutes. Ensure death by using decapitation, pithing, freezing, maceration, or other physical method as the second step when finfish have been rendered unconscious. Rapid chilling (0-4°C) until loss of orientation and cessation of opercular movements (10-20 seconds) and holding in ice water until death is assured. Use a screen divider to prevent direct contact with ice, but expose the entire fish to ice-cold water as quickly as possible. Hold fish in cold water for a minimum of 10 minutes after cessation of opercular movement.
Fish embryos up to 7 days post-fertilization (DPF)	<ul style="list-style-type: none"> Anesthetic overdose—Tricaine methane sulfonate* in a 0.05-1% solution (500 mg-10 g /L) for a minimum of 20 minutes. Ensure death by using decapitation, pithing, freezing, maceration, or other physical method as the second step. Rapid chilling (0-4° C) by holding in ice water for at least 20 minutes, followed by physical method, rapid freezing at -70°C, or immersion in diluted sodium or calcium hypochlorite (6.15%) to ensure death.

*Tricaine Methane Sulfonate (TMS or MS-222) is highly acidic and requires buffering with sodium bicarbonate, tris, NaOH, or other base to pH 7.4-7.5

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Animal Species	Method of Euthanasia
Birds	<ul style="list-style-type: none">▪ Overdose of anesthetic▪ Injection of Sodium Pentobarbital (euthanasia solution) IV; Intracoelomic, intracardiac and intraosseous injections can be used in unconscious or anesthetized birds.▪ Decapitation▪ CO₂ gas from compressed tank▪ Cervical dislocation on animals weighing less than 200gm (experienced handlers only)